An overview of stress response proteomes in Listeria monocytogenes

Soni, K A ; Nannapaneni, R ; Tasara, T

Abstract: Listeria monocytogenes adapts to diverse stress conditions including cold, osmotic, heat, acid, and alkali stresses encountered during food processing and preservation which is a serious food safety threat. In this review, we have presented the major findings on this bacterium's stress response proteomes to date along with the different approaches used for its proteomic analysis. The key proteome findings on cold, heat shock, salt, acid, alkaline and HHP stresses illustrate that the cellular stress responses in this organism are a culmination of multiple protein expression changes in response to a particular stress stimuli. Moreover, a number of key proteins may be involved in conferring the cross protective effects against various stress environments. As an example, ferritin-like protein (designated as Fri or Flp) is induced during cold, heat, and HHP stresses. Similarly, general stress protein Ctc is induced in cold and osmotic stresses while molecular chaperones such as GroEL and DnaK are induced in cold and heat stresses. Furthermore, a number of stress proteins also contribute towards L. monocytogenes virulence and pathogenicity. Future research may lead to understanding the stress proteomes of this pathogen induced on various food matrices and processing environments in which it can persist for long periods of time.

Originally published at:
Soni, K A; Nannapaneni, R; Tasara, T (2011). An overview of stress response proteomes in Listeria monocytogenes. Agriculture, Food and Analytical Bacteriology, 1(1):66-85.

AGRICULTURE, FOOD & ANALYTICAL BACTERIOLOGY

www.afabjournal.com

Agriculture, Food and Analytical Bacteriology

REVIEW

An Overview of Stress Response Proteomes in *Listeria monocytogenes*

Kamlesh A. Soni[1], Ramakrishna Nannapaneni[1*], and Taurai Tasara[2]

[1]Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS 39762, USA
[2]Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland

ABSTRACT

Listeria monocytogenes adapts to diverse stress conditions including cold, osmotic, heat, acid, and alkali stresses encountered during food processing and preservation which is a serious food safety threat. In this review, we have presented the major findings on this bacterium's stress response proteomes to date along with the different approaches used for its proteomic analysis. The key proteome findings on cold, heat shock, salt, acid, alkaline and HHP stresses illustrate that the cellular stress responses in this organism are a culmination of multiple protein expression changes in response to a particular stress stimuli. Moreover, a number of key proteins may be involved in conferring the cross protective effects against various stress environments. As an example, ferritin-like protein (designated as Fri or Flp) is induced during cold, heat, and HHP stresses. Similarly, general stress protein Ctc is induced in cold and osmotic stresses while molecular chaperones such as GroEL and DnaK are induced in cold and heat stresses. Furthermore, a number of stress proteins also contribute towards *L. monocytogenes* virulence and pathogenicity. Future research may lead to understanding the stress proteomes of this pathogen induced on various food matrices and processing environments in which it can persist for long periods of time.

Keywords: *Listeria monocytogenes*, proteome, cold stress, osmotic stress, heat stress, acid stress, alkali stress.

Agric. Food Anal. Bacteriol. 1: 66-85, 2011

INTRODUCTION

Listeria monocytogenes is an important food-borne pathogen with significant public health threats and economic impacts on the food industry. It causes "listeriosis" in humans, which is associated with a variety of symptoms ranging from flu-like illness to severe life threatening meningitis as well as high mortality (Lennon *et al.*, 1984). Epidemiological studies estimate that listeriosis to be responsible for approximately 19 % of food-related deaths in the United States annually (Scallan *et al.*, 2011). Suspected *L. monocytogenes* contamination is also among the leading causes of food recalls resulting in significant

Received: November 22, 2010, Accepted: April 9, 2011.
Released Online Advance Publication: April 1, 2011.
Correspondence: Ramakrishna Nannapaneni,
nannapaneni@fsnhp.msstate.edu.
Tel: +1 -662-325-7697 Fax: +1-662-325-8728

financial losses to the food industry due to the "zero tolerance" standard adopted for the ready-to-eat food products in the USA (Kramer *et al*., 2005; Marsden, 2001; Teratanavat and Hooker, 2004).

The prevalence of *L. monocytogenes* is mainly due to its wide-spread distribution and its ability to withstand adverse environmental conditions. This includes the ability of this pathogen to survive and grow at low temperatures, and resistance to high osmolarity, acidic and alkaline environments. Cold adaptation of this organism is of growing concern due to the changing life styles over the years that have increased the consumption of refrigerated and minimally processed food products. Besides cold storage, elevated salt concentrations are an alternative means of food preservation, but *L. monocytogenes* is also highly salt tolerant and has been documented to grow in the presence of as high as 10% NaCl (McClure *et al*., 1991). Jensen *et al.* (2007) recently showed that *L. monocytogenes* cells can display increased aggregation and biofilm formation when exposed to NaCl stress. Additionally this bacterium exhibits acid tolerance responses (ATR), which significantly increases its resistance to a subsequent lethal acid (pH 3.0-3.5) stress exposure after an initial encounter with the non-lethal acidic (pH 5.0-5.5) conditions. As an example, 4-log higher survival was observed in *L. monocytogenes* cells exposed to acid stress at pH 3.5 for 6 h after an initial 90 minute exposure to a mild acidic condition at pH 5.5 (Koutsoumanis *et al*., 2004). Similarly, *L. monocytogenes* may also acquire an increased alkaline stress tolerance subsequent to sublethal alkaline stress exposure (Mendonca *et al*., 1994). During food processing and preservation, *L. monocytogenes* cells may become exposed to multiple forms of sublethal stresses, leading to "stress hardening". Consequently, *L. monocytogenes* exposure to mild forms of particular stresses may inadvertently induce cross protection against subsequent exposures to lethal levels of other unrelated stresses. For example, it has been shown that acid (pH 4.5 for 1 h) or cold (10°C for 4 h) stressed *L. monocytogenes* LO28 (serotype 1/2c) cells tend to be more resistant to high hydrostatic pressure (HHP) in comparison to the non-stress adapted cells (Wemekamp-Kamphuis *et al*., 2002). Lou and Yousef (1997) reported that the heat stress of *L. monocytogenes* results in cell-hardening and subsequent osmoprotection and higher resistance of these cells to ethanol treatment. Likewise, *L. monocytogenes* cells were also found to be more thermotolerant after a combined acid and heat shock or after osmotic and heat shock treatments (Skandamis *et al*., 2008).

Stress adaptation events in *L. monocytogenes*, as in other microorganisms, includes coordinated induction of different stress protection systems within the affected cells. Proteomics and transcriptomics are both invaluable tools in delineation of the different mechanisms of stress response in microbes. Transcriptome analysis technologies while important in deciphering the global mRNA expression changes during stress responses, fail to capture all aspects of these molecular responses since mRNA transcripts changes may not directly correlate with protein expression due to the fact that transcripts produced in abundance may be rapidly degraded, translated poorly, or influenced through post-translational modifications. Therefore complementation of the transcriptome based analysis of stress responses with the proteome studies is important to get a clearer picture as proteins are the key functional units involved in physiological stress responses. As a result of new developments in microbial cell global protein profiling based on the protein identification approaches and bioinformatics, researchers are now also able to monitor and determine the importance of stress induced proteins in *L. monocytogenes* during its adaptation to diverse conditions. A number of proteome profiling studies performed on this organism so far have already provided extensive preliminary insights into gene and protein expression changes that are associated with the environmental stress adaptation in this bacterium. The purpose of this review is to discuss the significant developments in proteomic analysis of the stress-adaptation in *L. monocytogenes* with focus on cold, heat, osmotic, acid, alkaline, and HHP adaptation along with cross linking between stress proteins and virulence.

PROTEOMIC TECHNOLOGIES APPLIED IN *L. MONOCYTOGENES* ANALYSIS

The summary of different gel-based and non-gel based techniques and their assay principles are discussed in-depth in recent review articles by Haynes *et al.* (2007) and Nesatyy and Suter (2007). For *L. monocytogenes*, the most commonly applied protocols to date have used two-dimensional gel electrophoresis (2DE) for protein separation (Folio *et al.*, 2004; Mujahid *et al.*, 2007; Ramnath *et al.*, 2003; Schaumburg *et al.*, 2004). The majority of the *L. monocytogenes* stress proteome studies utilized soluble cellular proteins (excluding the extracellular fraction) that were fractionated using mechanical disruption alone or protocols combining mechanical disruption and enzymatic lysis. In the earlier studies most of the proteins were not identified (; Bayles *et al.*, 1996; Phan-Thanh and Gormon, 1995), although in later studies a significant number of the 2DE separated proteins were identified by mass spectrophotometry (MS) (Abram *et al.*, 2008b; Dumas *et al.*, 2008; Folio *et al.*, 2004; Mujahid *et al.*, 2007; 2008; Phan-Thanh and Jansch, 2006; Schaumburg *et al.*, 2004). More recently however, non-gel based approaches that combine liquid chromatography (LC) separation and MS (LC-MS/MS) are increasingly used. The fractionated complex bacterial protein mixtures are digested into peptides, separated by liquid chromatography and analyzed in MS, taking advantage of the advances in bioinformatics to identify even larger numbers of the fractionated proteins (Abram *et al.*, 2008b; Calvo *et al.*, 2005; Trost *et al.*, 2005).

The majority of studies that have compared protein expression between normal versus stress exposed *L. monocytogenes* cells using 2DE gel-based protein separation with or without subsequent application of MS to identify separated proteins (Bayles *et al.*, 1996; Duche *et al.*, 2002a; Esvan *et al.*, 2000; Phan-Thanh and Gormon, 1995; 1997; Phan-Thanh and Mahouin, 1999; Wemekamp-Kamphuis *et al.*, 2004a). A 2DE reference map covering an estimated 28.8% of potential gene products was generated from the soluble subproteome of *L. monocytogenes* EGDe serotype 1/2a strain (Folio *et al.*, 2004). Ramnath *et al.* (2003) also used this approach and detected two proteins found in *L. monocytogenes* EGDe but were absent in some food isolates. The identification of these proteins revealed they were involved in glycolytic pathway and metabolism of coenzymes, but the relevance of their differential expression specifically in such food isolates remains unknown. The drawbacks of gel based 2DE proteomics include poor reproducibility in separation of highly basic or hydrophobic proteins, gel-to-gel variations and poor resolution of high molecular weight protein complexes. Attempts to overcome these drawbacks include the recent use of 2D-DIGE (Two dimensional-difference gel electrophoresis) based proteomics analysis. By using different fluorescent dyes such as Cy2, Cy3 or Cy5 for protein labeling, such approaches allow protein mixtures of different origins to be analyzed within the same gel run. Thus these approaches are more amenable to stress proteome response studies where protein expression patterns of stress-adapted cells and control samples can be directly compared within the same gel run to minimize the influence of gel-to-gel variations. Folsom and Frank (2007) used a 2DE-DIGE based proteomics approach to analyze protein expression changes associated with chlorine resistance and biofilm formation in a hypochlorous acid tolerant variant of the *L. monocytogenes* Scott A (4b) strain. They found 19 proteins that were differentially expressed between planktonic and biofilm cells of a hypochlorous acid tolerant cultural variant of this strain (Folsom and Frank, 2007). Six of these differentially expressed proteins were subsequently identified by peptide-mass mapping. They included three ribosomal proteins (L7, L10 and L12), peroxide resistance protein (Dpr/Flp/Fri), sugar-binding protein (Lmo0181), and a putative protein Lmo1888 of yet unknown function. This study also revealed that peroxide stress resistance proteins Fri that is involved in multitude of other stresses was expressed 2.2-fold times higher in biofilm than in planktonic cells. At phenotypic level it was observed that *L. monocytogenes* cells present in biofilm mass were more resistant to sanitization treatments compared to planktonic cells (Pan *et al.*, 2006). Although not yet widely adapted for *L. monocytogenes* analysis, LC

based techniques seem capable of detecting even higher numbers of proteins compared to traditional 2DE gel-based techniques. As an example when the same protein fraction of cell free supernatant (extracellular) of *L. monocytogenes* EGDe was analyzed, 105 proteins were identified using LC-MS/MS compared to 58 detected by 2DE (Trost *et al.*, 2005). Forty-five of the detected proteins were found to be common between the two methods. The analysis of differential protein expression between *L. monocytogenes* 10403S and its σ^B null mutant strain using the LC-MS/MS with iTRAQ (isotope tag for relative and absolute quantification) identified 35 σ^B regulated proteins, whereas the 2DE approach only managed to detect 13 proteins. Four proteins were common between the two methods (Abram *et al.*, 2008b). A combination of SDS-PAGE and LC-MS/MS detected 301 membrane associated proteins of *L. monocytogenes* EGDe (Wehmhoner *et al.*, 2005). This was greater than 79 proteins detected using the 2DE approach by Mujahid *et al.* (2007). One possible reason for increased protein detection with SDS-PAGE/LC-MS/MS might be increased protein solubilization in the SDS-PAGE sample buffer in comparison to the urea based sample buffer applied in the 2DE-MS approach (Haynes and Roberts, 2007).

In another example LC-LC-MS/MS combination also called the multidimensional protein identification technique (MudPIT), has also been used for proteome analysis of *L. monocytogenes* cells. Fifteen proteins that covalently bound the LPXTG motif were identified in the subproteome fraction of cell wall associated proteins of *L. monocytogenes* strain EGDe (Calvo *et al.*, 2005). The SrtA and SrtB enzymes anchor surface proteins to the cell wall. Surface proteins recognized by these two sortases were also analyzed using LC-LC-MS/MS in the EGDe strain. A total of 13 and 2 LPXTG-containing proteins were identified in *srtA* and *srtB* null mutant strains (Pucciarelli *et al.*, 2005). Recently, MudPIT was used to study the differences that exist between serotype 1/2a (strain EGD) and 4b (strain F2365) (Donaldson *et al.*, 2009). In total, 1754 EGD proteins and 1427 F2365 proteins were detected representing 50-60% of total *Listeria* proteome coverage. In total 1077 proteins were common to both serotypes and of these 413 proteins displayed significantly differential expression level between the two serotypes.

PROTEOME ANALYSIS IN STRESS-ADAPTED *L. MONOCYTOGENES* CELLS

The ability of *L. monocytogenes* to sense and respond to a particular stress factor has implications for both survival and virulence properties of this bacterium. Stress exposure elicits various fundamental changes in this organism's cellular physiology. These changes are mediated via multiple and specific changes in gene and protein expression profiles in cells. Proteins associated with cold, heat, osmotic, acid, and high hydrostatic pressure stress adaptation will be discussed in the following sections.

Cold stress adaptation

The growth of *L. monocytogenes* on cold preserved food products is one of its important food safety challenges. In addition to decreased metabolic capacity, cold stress exposed microorganisms are faced with a wide range of structural and functional impediments in membrane structures, nucleic acids (DNA and RNA), and macromolecular assemblies such as ribosomes (Schumann, 2009). The putative integral membrane protein PgpH, whose deletion leads to impaired cold growth, has been proposed as a possible cold sensing factor in *L. monocytogenes* (Liu *et al.*, 2006). Based on the proposed model, environmental cold stress sensed through membrane bound PgpH proteins is conveyed intracellularly through homeodomain dependent signaling pathways.

Using 2DE gel-based proteome analysis, initial studies revealed modulation in expression of between 10 to 38 proteins in association with cold stress adaptation of this organism (Bayles *et al.*, 1996; Hebraud and Guzzo, 2000; Phan-Thanh and Gormon, 1995). Of these differentially expressed proteins visualized, the predominating cold shock protein was subsequently identified through microsequencing as ferritin (Fri) (designated as Flp or Fri) (Hebraud and

Guzzo, 2000). The role of this protein in cold adaptation was also later phenotypically confirmed when Dussurget *et al.* (2005) created a *fri* null mutant strain in *L. monocytogenes* EGDe, which exhibited a cold sensitive phenotype. Although physiological and cold adaptation roles of Fri are not yet well understood, it is hypothesized that it might facilitate alleviation of oxidative stress environments developing in cold stress exposed *L. monocytogenes* cells (Liu *et al*., 2002; Tasara and Stephan, 2006). Wemekamp-Kamphuis *et al.* (2002) described four approximately 7 kDa protein that were cold inducible in *L. monocytogenes* LO28 as determined by using a combination of 2DE gel electrophoresis and immunoblotting. These proteins designated Csp1-Csp4, were described as the *L. monocytogenes* cold shock family proteins based on their cross reactivity with anti-*B. subtilis* CspB polyclonal antibodies. Although their identity as such was not confirmed by peptide mass fingerprinting (PMF) in this work, genomic information show that *L. monocytogenes* harbors three proteins of the cold shock domain protein family (Glaser *et al*., 2001). Two of these *L. monocytogenes* Csp proteins, CspL (CspA) and CspD have now been confirmed to be functionally vital for efficient cold growth in this bacterium (Schmid *et al*., 2009). CspA and CspD proteins, based on knowledge from other microorganisms, are also presumed to facilitate cold growth possibly through nucleic acid (DNA and RNA) chaperone-like functions (Horn *et al*., 2007). This facilitates DNA replication and gene expression events that may otherwise be hampered through secondary structures that tend to form in bacterial cells at low temperatures.

Meanwhile, a more comprehensive cold adaptation proteome analysis in this bacterium has been recently described. Cacace *et al*. (2010) performed detailed proteome analysis on *L. monocytogenes* cells grown for 13 days at 4°C with subsequent MALDI (Matrix-assisted laser desorption/ionization) analysis. Proteome analysis revealed that 57 proteins in total were over-expressed and eight were repressed in cold grown cells compared to cells cultivated at 37°C. Proteome changes detected in this study indicated the increased synthesis of proteins linked to energy production, oxidative stress resistance, nutrient uptake, lipid synthesis, and protein synthesis and folding. Cold stress adaptation proteins identified by this study that are of particular interest include: OppA, Ctc, GroEL and DnaK. The OppA protein, which facilitates accumulation of short peptide substrates, is important for efficient cold growth in this bacterium and at phenotypic level *oppA* null mutant of this bacterium was unable to grow at low temperature (5°C) (Borezee *et al*., 2000). Ctc is a general stress protein which has been found to promote the adaptation of *L. monocytogenes* cells to high osmolarity conditions (Gardan *et al*., 2003b). The GroEL and DnaK proteins are molecular chaperones that promote proteins refolding and degradation of stress damaged proteins that accumulate under different suboptimal conditions including heat stress (Sokolovic *et al*., 1990). The cold growth associated induction of the Ctc, GroEL and DnaK proteins, which have been previously associated with adaptation to other stresses (i.e. Ctc for cold and osmotic stress and GroEL-DnaK for cold and heat stress) conditions may thus indicate commonality of some stress adaptive responses in this bacterium (Cacace *et al*., 2010; Duche *et al*., 2002a,b; Gardan *et al*., 2003b; Sokolovic *et al*., 1990).

The accumulation of compatible solutes especially glycine, betaine, and carnitine also promotes cold growth in various bacteria including *L. monocytogenes* (Mendum and Smith, 2002; Smith, 1996; Wemekamp-Kamphuis *et al*., 2004b). There are no enzymatic systems for the de novo synthesis of main cryoprotective compatible solutes glycine, betaine, and carnitine in *L. monocytogenes*, but transport systems (Gbu, BetL and OpuC) that accumulate them from environmental sources are present, and deletion of genes coding for these transporters has confirmed that they facilitate efficient cold growth of this bacterium (Angelidis *et al*., 2002; Ko and Smith, 1999; Sleator *et al*., 1999). Analysis of cold-sensitive mutants in which Lmo1078 (Chassaing and Auvray, 2007), and LtrC (Chan *et al*., 2007) proteins are inactivated also indicates that these proteins functionally contribute to cold adaptation processes in *L. monocytogenes*. The Lmo1078 protein is a UDP-

glucose pyrophosphorylase proposed to promote cold adaptation through enhanced UDP-glucose production at low temperatures. UDP glucose is an essential substrate in lipoteichoic acid production and might facilitate maintenance of architectural integrity in cell wall and membrane structures leading to protection of bacterial cells from cold stress damage (Chassaing and Auvray, 2007).

Heat stress adaptation

The understanding of heat stress adaptation in food-borne pathogens is an important issue since heating constitutes one of the major food processing and preservation methods. The heat shock response is one of the most universal and extensively studied physical stress responses in living organisms. This process involves increased production of various cell protective protein systems, which ultimately promotes general environmental stress resistance and enhanced thermal tolerance (Gandhi and Chikindas, 2007; Klinkert and Narberhaus, 2009; Muga and Moro, 2008; van der Veen *et al.*, 2007). Similar to other bacteria, *L. monocytogenes* synthesizes a highly conserved set of proteins, also defined as heat shock proteins (Hsps), upon exposure to high temperatures (>45°C). Hsps include highly conserved molecular chaperones and proteases that functionally prevent nonproductive protein aggregations under different stress environments. GroEL and DnaK are major Hsps that promote refolding and degradation of damaged proteins through ATP-dependent mechanisms (Kandror *et al.*, 1994; Sherman and Goldberg, 1996; van der Veen *et al.*, 2007). These two proteins are highly conserved among living organisms and also constitute as the main Hsp chaperones observed in *L. monocytogenes* (Gahan *et al.*, 2001; Hanawa *et al.*, 2000)

Using proteome analysis, the induction of up to 15 Hsps in response to heat shock (48°C/30 min) was observed using SDS-PAGE (Sokolovic *et al.*, 1990). Of these, two Hsps were identified as GroEL and DnaK in *L. monocytogenes* CLIP 54149 (serotype 1/2a) based on immunological detection. In another study the induction of as many as 32 Hsps was observed using preparative 2DE gels of *L. monocytogenes* EGD in response to a temperature shock of 49°C/15 min (Phan-Thanh and Gormon, 1995). One identified predominant protein, Fri, with molecular weight 18 kDa and pI of 5.1 showed 50.6-fold inductions due to heat shock. This very same protein spot was 10.5-fold induced in response to cold shock (Phan-Thanh and Gormon, 1995). Similarly, other researchers have also observed the transcriptional induction of *fri* transcripts in response to heat (Hebraud and Guzzo, 2000; van der Veen *et al.*, 2007) and cold stress (Dussurget *et al.*, 2005). Phenotypically *fri* gene null *L. monocytogenes* EGDe cells also failed to reach the maximal optical density compared to the wild type strain during growth under heat at 45°C (Dussurget *et al.*, 2005). These findings together suggest that ferritin-like protein is important for high and low temperature adaptation in *L. monocytogenes*. Recently, Agoston *et al.* (2009) compared the effect of mild and prolonged heat treatments on *L. monocytogenes* cells using 2DE analysis. In line with the reduced metabolic activity at suboptimal temperature, large numbers of metabolic proteins were suppressed during heat exposure in this study which is also consistent with the observation from other studies (Phan-Thanh and Gormon, 1995; Phan-Thanh and Jansch, 2006). Importantly, *L. monocytogenes* stress protein DnaN, a beta subunit of polymerase III, was highly induced in response to different heat shock treatments. Observed induced expression of DnaN, involved in DNA synthesis process, may indicate its role in increased synthesis of some HSPs.

Osmotic stress adaptation

The osmotolerance of *L. monocytogenes* is another property crucial to survival and growth of this pathogen at high salt levels and low water activity environments encountered in conserved food products. Osmotic stress adaptation in microorganisms depends on the modulation of both ionic and organic solute pools so as to sustain cytoplasmic water and turgor pressure at levels, which are compatible with cell viability and growth at low water activity (Booth and Louis, 1999; Wood, 2007).

L. monocytogenes cells cope with elevated levels of osmolarity through appropriate changes in protein expression levels. Significant modulation (approximately 32 proteins) in protein expression under hyper osmotic conditions (3.5% to 6.5% NaCl concentration) was first visualized in preparative 2DE gels (Esvan *et al.*, 2000) and some of the salt stress adaptation proteins were later identified (Duche *et al.*, 2002a,b). Identified osmotic stress proteins include those related to general stress (Ctc and DnaK), transporters (GbuA and AppA), ribosomal proteins (RpsF, 30S ribosomal protein S6), as well as proteins involved in general metabolism processes (Ald, CcpA, CysK, TufA (EF-Tu), Gap, GuaB, PdhA, PdhD, and Pgm) (Duche *et al.*, 2002a,b). Among the salt stress induced proteins, the role of Ctc in osmotolerance was further characterized by Gardan *et al.* (2003b), who demonstrated that *ctc* gene is involved in *L. monocytogenes* osmotolerance. They found that growth of the *ctc* mutant strain was significantly impaired compared to its isogenic wild type *L. monocytogenes* LO28 strain in minimal medium with 3.5% NaCl.

Other than the differential expression of salt stress proteins, increased uptake of glycine betaine and carnitine osmolytes via *betL*, *gbu*, and *opuC* encoded transporter porters is crucial under hyper-osmotic conditions. Accumulation of these osmolytes prevents the intracellular water loss by counteracting external osmolarity and keeping the macromolecular structure of the cells intact. Indeed, the induced expression (>2-fold) of GbuA transporter protein under high osmolarity (3.5% NaCl) has been observed in 2DE analysis of *L. monocytogenes* LO28 (Duche *et al.*, 2002a). Meanwhile the induction of compatible solute transporter encoding genes, *betL*, *gbu*, and *opuC* in response to higher osmolarity has been reported at the transcriptional level in *L. monocytogenes* cells (Fraser *et al.*, 2003). Interestingly these transporter systems expressed under hyper-osmotic stress conditions are the same as the ones expressed under cold stress (Mendum and Smith, 2002; Smith, 1996; Wemekamp-Kamphuis *et al.*, 2004b), suggesting that some of the mechanisms counteracting osmotic and cold stress may be common in *L. monocytogenes*. Moreover, the cold shock protein CspD also facilitates both osmotic and cold stress adaptation in *L. monocytogenes* and a mutant strain lacking *cspD* gene also display a stress sensitive phenotype under NaCl salt stress conditions (Schmid *et al.*, 2009). Other important proteins in *L. monocytogenes* salt stress adaptation are HtrA (Wonderling *et al.*, 2004) and Lmo 1078 (Chassaing and Auvray, 2007). The HtrA protein is a general stress response serine protease that contributes to osmotic stress adaptation functions through its role in degradation of salt stress damaged proteins. At the phenotypic level the *L. monocytogenes htrA* null mutant displays diminished growth in presence of NaCl stress. The Lmo1078 promotes both cold and osmotic tolerance based on its proposed functional contribution to maintenance of cell wall and membrane architectural integrity in this bacterium. The CstR transcriptional repressor protein is also involved in modulation of *L. monocytogenes* osmotic stress tolerance functions since a CstR null mutant of this bacterium displays improved growth under NaCl salt stress conditions (Nair *et al.*, 2000b).

Acid stress adaptation

The adaptation of microorganisms to acid stress environments includes significant gene and protein expression changes associated with, among other response, the mobilization of cellular mechanisms that consume acids and generate basic amines (Foster, 2004; Merrell and Camilli, 2002). *L. monocytogenes* cells face acid stress conditions in low pH foods and at various stages during human infection. *L. monocytogenes* counteracts acidic stress conditions by production of various acid stress response proteins (ASPs). ASPs were initially designated based on their location on the preparative 2DE gels (Davis *et al.*, 1996; O'Driscoll *et al.*, 1997) and some were later identified by PMF (Phan-Thanh and Mahouin, 1999; Wemekamp-Kamphuis *et al.*, 2004a). Some of the identified ASPs include: proteins involved in respiration (enzyme dehydrogenases and reductases), osmolyte transport (GbuA), protein folding and repair (Chapronin, GroEL, ClpP), general stress re-

sponse (sigma H homologous of *B. subtilis*), flagella synthesis (FlaA), and metabolism (Pfk, GalE) (Phan-Thanh and Mahouin, 1999; Wemekamp-Kamphuis *et al*., 2004a).

The acid tolerance response (ATR) is characterized by increased microbial cell resistance to lethal acid after an exposure to mild acidic condition (Koutsoumanis *et al*., 2004). This phenomenon has been examined by a number of studies in *L. monocytogenes* cells. When the synthesis of ASPs in *L. monocytogenes* LO28 produced under both mild (pH 5.5 for 2 h) and lethal (pH 3.5 for 15 min) acidic conditions were compared to a normal pH (~7.2), a total of 37 proteins were induced under mild acidic treatment and 47 under lethal acidic treatment, with 23 of the induced proteins being common under both conditions (Phan-Thanh and Mahouin, 1999). The different aspects of acid stress adaptive mechanisms in *L. monocytogenes* are well elucidated from acid stress adaptation mechanisms studies in this bacterium (Abram *et al*., 2008a; Ferreira *et al*., 2003; Phan-Thanh and Jansch, 2006; Ryan *et al*., 2008b). In brief, when exposed to a lower external pH, bacterial cells attempt to maintain their cytoplasmic pH by decreasing the membrane permeability to protons, buffering their cytoplasm, and by equilibrating the external pH through catabolism (Phan-Thanh and Jansch, 2006). One of the ways that limit the bacterial permeability to proton is through changes in the lipid bilayer of cell membrane. Giotis *et al*. (2007) suggested that there was an increased concentration of straight chain fatty acids and decreased concentration of branched chain fatty acids in *L. monocytogenes* 10403S cells grown under acidic conditions (pH 5.0 to 6.0) compared to neutral pH. Another important approach that the bacterial cells use for dispelling the protons outside the cells is to accelerate electron transferring reactions through enhanced oxidation reduction potential. The ASPs identified as dehydrogenases (GuaB, PduQ and lmo0560) and reductases (YcgT) together with respiratory enzymes are implicated to play an important role in maintaining pH homeostasis by active proton transport (Phan-Thanh and Jansch, 2006).

Organic acid salts such as sodium lactate and sodium diacetate are extensively used in ready-to-eat (RTE) meat products as anti*Listeria*l food preservatives. Recently Mbandi *et al*. (2007) used 2DE to evaluate the protein induction in *L. monocytogenes* Scott A by these organic salts. Experiments were conducted in defined medium with either sodium lactate (2.5%) or sodium diacetate (0.2%) or in combination. Some of the proteins that showed substantial up or down regulation (>10 fold) were identified using PMF. Oxidoreductase and lipoproteins were upregulated whereas DNA-binding proteins, alpha amylase and SecA were repressed during exposure to these organic acid salts. Identified enzyme protein oxidoreductase in *L. monocytogenes* has been previously suggested to be involved in dispelling proton molecules to maintain cell homeostasis (Phan-Thanh and Jansch, 2006).

The glutamate decarboxylase (GAD) and arginine deiminase (ADI) are well described major acid adaptive mechanisms in *L. monocytogenes*. *L. monocytogenes* LO28 strain with a mutation in genes of GAD proteins GadA, GadB and GadC displayed higher acid stress sensitivity in an acidified reconstituted skim milk background (Cotter *et al*., 2001b) and gastric fluid (Cotter *et al*., 2001a). The *L. monocytogenes* ADI system includes proteins ArcA, ArcB and ArcC and ArcD for the conversion and transfer of arginine into ornithine and deletion in functional genes of ADI leads impaired growth in mildly acidic conditions (pH 4.8) and survival in lethal pH conditions (pH 3.5) (Ryan *et al*., 2009).

Alkaline stress adaptation

L. monocytogenes cells are more resistant to alkaline stress in comparison to other foodborne pathogens such as *Salmonella* Enteritidis and *E. coli* O157:H7 (Mendonca *et al*., 1994). At pH 12, *L. monocytogenes* F5069 (serotype 4b) cell concentrations decreased by only 1-log in 10 min compared to 8-log reductions observed for *E. coli* and *S.* Enteritidis within 15 s. Earlier, 2DE analysis of alkaline stressed (pH 10.0 for 35 min) *L. monocytogenes* EGDe cells by Phan-Thanh and Gormon (1997) showed induction of 16 proteins, synthesis of 11 novel proteins, and

repression of nearly half of the total proteins in comparison to non-stressed cells. Recently, Giotis *et al.* (2008) also reported the repression of a large number of proteins along with synthesis of 8 novel proteins in response to alkaline stress of *L. monocytogenes* 10403S strain. In addition to proteomic analysis, they also evaluated the alkaline stress adaptive mechanism using microarray transcriptional profiling and found 390 gene transcripts differentially expressed (Giotis *et al*., 2008). Protein identification of four differentially expressed proteins by peptide-mass mapping revealed induction of heat shock proteins DnaK and GroEL and repression of DdlA (alanine ligase) and AtpD (ATP synthase). These identified proteins spots were also found to be induced or repressed in microarray analysis. In addition, screening library of Tn*917- lac* insertional mutants in *L. monocytogenes* LO28 identified 12 mutants sensitive to alkaline conditions, though identification of transposition target suggest they all carried mutations in only putative transporter genes (Gardan *et al.*, 2003a).

High hydrostatic pressure (HHP) stress adaptation

L. monocytogenes cells undergo mechanical stress following HHP treatment. The usual pressure range employed in HHP is in the range of 200-600 MPa for 5-10 min depending on the food matrices. Such high pressure damages the cell membrane and results in leakage of cell content along with dissociation of protein complexes (Gross and Jaenicke, 1994). However, HHP treated *L. monocytogenes* cells were found to be sublethally injured with their metabolic-activity largely maintained and had the potential for a gradual recovery (Ritz *et al.*, 2006). In addition although *L. monocytogenes* cells in HHP treated cooked ham displayed a lag phase lasting up to 1.5 months, they subsequently recovered to grow more than 5-logs over 3 months (Aymerich *et al.*, 2005).

To characterize the HHP induced proteins enabling resistance to mechanical stress, Jofre *et al.* (2007) conducted 2DE analysis of *L. monocytogenes* CTC1011 (serotype 1/2c) after treatment with 400 MPa for 2 h and observed expression of 23 proteins being modulated. These high pressure induced proteins were related to ribosomal function (RplJ, RplL, RpsF, RpsB, IleS, GatA), transcription (GreA), protein degradation (PepF, PepT), protein folding (GroES), metabolism (PflB, Pta, Zwf, Ald,), general stress (Fri) and unknown functions. Of these high pressure induced proteins, chaperone GroES may be necessary in refolding of dissociated protein complexes following HHP treatment, and peptidases (PepF, PepT) may contribute to degradation of proteins that cannot be folded by molecular chaperones. Flp has been previously elucidated to have roles in cold, heat, and oxidative stress adaptation (Dussurget *et al.*, 2005). Moreover, *L. monocytogenes* shows increased resistant to HHP treatment following prior exposure to cold stress along with induced expression of cold shock proteins following HHP treatment.

Implications of *L. monocytogenes* stress adaptation to virulence responses

The stress responses of *L. monocytogenes* are not only important in survival of hostile external and food-associated environments but also during host colonization processes. The pathogenicity of food-borne *L. monocytogenes* also depends on their physiological status at infection, which is determined by, among other factors, the environmental stress challenges encountered and stress responses activated prior to interaction with susceptible hosts. Besides the fact that acid stress adaptation of this bacterium promotes survival in acidic food environments, this process has been also shown to modulate various aspects of virulence in this pathogen. As an example, the pathogenic potential of this bacterium can be increased through improved viability in the gastrointestinal tract, which includes increased survival of the gastric acid stress challenges. The increased expression of virulence genes as well as enhanced cell adhesion and invasion has been reported in association with acid stress adaptation of *L. monocytogenes* cells (Conte *et al.*, 2000; Garner *et al.*, 2006; Olsen *et al.*, 2005; Werbrouck *et al.*, 2009). Conte *et al.* (2000) detected enhanced Caco-2 cell

invasion ability, in addition to improved survival and proliferation in activated murine macrophages of *L. monocytogenes* cells preadapted by mild organic acid stress exposure. Werbrouck *et al.* (2009) also described increased cellular invasiveness and inlA mRNA levels in their analysis of acid stress adapted *L. monocytogenes* cells. In similar fashion there was an increased transcription of virulence genes such as *prfA*, *inlA* and *inlB*, as well as enhanced adhesion and invasion of Caco-2 cells in two *L. monocytogenes* strains adapted to prolonged acid stress (Olesen *et al.*, 2009). Another stress commonly encountered by *L. monocytogenes* cells in food associated environments considered to potentially influence virulence of this bacterium is NaCl osmotic stress. NaCl stress exposure is associated with increased expression of various general stress resistance and virulence genes in this bacterium suggesting that osmotic stress adaptation events along the food supply chain may enhance subsequent pathogenicity (Kazmierczak *et al.*, 2003; Olesen *et al.*, 2009; Sue *et al.*, 2004). Phenotypically increased cell adhesion and invasion *in vitro* has been observed in NaCl stress adapted *L. monocytogenes* cells (Garner *et al.*, 2006; Olesen *et al.*, 2009). The significance of these phenotypic observations however remains to be further examined. One study, which examined the growth of some food environment persistent strains and clinical isolates under NaCl osmotic stress, was not able to detect significant influence of this stress exposure on pathogenicity of these strains using several virulence models (Jensen *et al.*, 2008). Similarly, Wałecka *et al.*, (2011) did not find increased expression of internalins with salt stress and suggested that bacterial growth phase instead of salt stress was direct determinant of *L. monocytogenes* invasiveness. Hence the above mentioned reports determining the involvement of salt stress show conflicting findings and more work in this direction would be required to understand the factors that result in such differing view. The expression of *prfA* controlled virulence genes and cell invasion capacity of *L. monocytogenes* cells is temperature dependent and pathogenicity in some meat-processing plant derived strains of this bacterium was reported to decrease during long term cold storage at 4°C (Duodu *et al.*, 2010; Johansson *et al.*, 2002; McGann *et al.*, 2007). Similarly, cold stress exposed wild type and mutants lacking csp genes in the *L. monocytogenes* EGDe strain were significantly impaired in cell invasion relative to corresponding controls grown at 37°C (Loepfe *et al.*, 2010). Temperature dependent virulence gene expression repression as well as membrane damage and cell surface modifications in these organisms exposed at low temperatures might lead to phenotypic virulence defects observed in cold adapted *L. monocytogenes* organisms.

Van de Velde *et al.* (2009) compared proteomes between *L. monocytogenes* cells grown in human THP-1 monocytes versus those growing extracellularly in TSB broth using 2D-DIGE. Down regulations of general stress protein Ctc and oxidative stress protein Sod was detected suggesting that compared to extra cellular environment the intracellular uptake by host cells may be more favorable environment for *L. monocytogenes* survival and adaptations. Shin *et al.* (2010) observed the increased σ^B activity, as measured by ß-galactosidase *lacZ* promoter assay, to vancomycin antibiotic stress. While subsequent proteome analysis of *L. monocytogenes* σ^B wild type and null mutant strains using LC-ESI-MS/MS also revealed among other proteins the increased production of the virulence protein InlD. Fri protein is another general stress response protein with virulence promoting functions in *L. monocytogenes*. It has been shown by using both mice challenge and macrophage cell virulence models that *fri* null strains of *L. monocytogenes* are significantly impaired (Dussurget *et al.*, 2005; Mohamed *et al.*, 2006; Olsen *et al.*, 2005). Proteome analysis of the *fri* mutant and wild type strain was compared to reveal repression in Hly (Listeriolysin O) and stress response proteins CcpA (Catabolite control protein A) and OsmC (Dussurget *et al.*, 2005).

The stress induced chaperone proteins ClpB, ClpC, ClpE, ClpP have all been shown to provide virulence promoting activities in *L. monocytogenes* and thus it is possible that their induction in this bacterium in response to stress in food associated environments also increases the capacity of stress

adapted organism to survive hostile host environments as well as enhance their pathogenicity (Chastanet *et al.*, 2004; Gaillot *et al.*, 2000; Nair *et al.*, 1999; 2000a;). Meanwhile Olesen *et al.* (2009) in their recent study showed that acid exposed *L. monocytogenes* cells displaying increased Caco-2 cell virulence also displayed increased expression of genes encoding the ClpC and ClpP. The RNA binding regulatory protein Hfq, is another general stress response modulating protein which has been shown to protects cells from osmotic and ethanol stress as well as facilitate enhanced pathogenicity in *L. monocytogenes* infected mice (Christiansen *et al.*, 2004). Stack *et al.* (2005) found that the HrtA serine protease, which protects *L. monocytogenes* from various stresses including exposure to acidic conditions also contributes towards virulence capablities of this bacterium. The general stress response protein σ^B, which facilitates *L. monocytogenes* adaptation to multiple stresses has also been shown to promote virulence and cell invasiveness in this bacterium (Garner *et al.*, 2006; Ivy *et al.*, 2010). Recently it was shown that the importance of σ^B responses in these aspects might be lineage specific with its activity being important in lineage I, II, IIIB strains but not in IIIA (Oliver *et al.*, 2010).

Role of alternative sigma factor (σ^B) in *L. monocytogenes* stress adaptation

In *L. monocytogenes*, σ^B is a major stress response regulator and mutant strain lacking σ^B activity shows increased sensitivity to a wide range of stresses including cold (Becker *et al.*, 2000; Chan *et al.*, 2007; 2008; Moorhead and Dykes, 2004; Raimann *et al.*, 2009; Wemekamp-Kamphuis *et al.*, 2004a;), heat (Hu *et al.*, 2007a,b; van der Veen *et al.*, 2007), osmotic (Becker *et al.*, 1998, Fraser *et al.*, 2003; Okada et al 2008; Raimann *et al.*, 2009), acid (Cotter *et al.*, 2001a,b; Ryan *et al.*, 2008a; Wemekamp-Kamphuis *et al.*, 2004a), and HHP (Wemekamp-Kamphuis *et al.*, 2004a). The main role of σ^B in *L. monocytogenes* is to regulate the expression of various stress response associated genes. As an example, Flp is a general stress protein involved in cold, oxidative and heat stress adaptation. The expression of *fri* gene encoding Flp protein is partially regulated through σ^B-dependent pathways in *L. monocytogenes* 10403S (Chan *et al.*, 2007).

To identify the proteins that show σ^B dependent expression in the acidic conditions, 2DE analysis of acid adapted (pH 4.5) and non-adapted cells (both wild type and σ^B mutant) was performed (Wemekamp-Kamphuis *et al.*, 2004a). The expression of 9 proteins was dependent on σ^B during acid stress and some of these proteins were identified using PMF. The identified proteins with σ^B dependent expression in response to HHP stress included Pfk, GalE, ClpP, and Lmo1580. The Pfk (6-phosphofructokinase) and GalE are enzymes involved in glycolysis and sugar metabolism, respectively, and ClpP is the ATP-dependent chaperone protease that plays a role in preventing the accumulation of misfolded proteins. The induction of ClpP protein expression may be necessary in acidic conditions to help in resolution of protein aggregations that are likely to occur due to acid stress induced protein damage.

Recently, the role of σ^B regulon on *L. monocytogenes* 10403S cells grown to stationary phase in the presence or absence of 0.5 M NaCl was evaluated using both 2DE and iTRAQ (Abram *et al.*, 2008b). Using a combination of these two approaches a total of 38 proteins (17 induced and 21 repressed) were identified whose expression was σ^B dependent. Among these σ^B controlled proteins, 10 proteins (7 positively regulated and 3 negatively regulated by σ^B) were further classified based on their potential role in stress related functions. Of these 7 σ^B positively regulated proteins, two proteins OpuC and HtrA were previously conferred to have role in *L. monocytogenes* stress adaptation (Fraser *et al.*, 2003; Wonderling *et al.*, 2004). OpuC is involved in osmolyte transfer needed for osmotic and cold adaptation (Fraser *et al.*, 2003) and HtrA serves as a protease whose deletion leads to growth defects under NaCL stress (Wonderling *et al.*, 2004). Intracellular accumulation of glycine betaine and carnitine osmolytes is necessary in cold

as well as osmotic stress. Expressions of the osmolyte transporter proteins, Gbu and Opu, have been shown to be at least partially dependent on the σ^B activity (Cetin *et al.*, 2004; Fraser *et al.*, 2003;). Also *L. monocytogenes* 10403S strain with a null mutation in the σ^B gene showed substantial defects in its ability to accumulate glycine betaine and carnitine osmolytes (Becker *et al.*, 1998; 2000). Moreover, σ^B deletion impairs the ability of *L. monocytogenes* 10403S cells to withstand against heat stress (55°C for 30-60 min) and class II heat shock genes, which also includes the osmolyte transporter gene *opuC*, are positively upregulated following heat shock (48°C for 3 min) in *L. monocytogenes* EGDe strain (Hu *et al.*, 2007a; van der Veen *et al.*, 2007). Using transcriptional analysis, Ryan *et al.* (2008a) reported the induction of the σ^B in response to sublethal levels of detergent stress. In addition, following HHP treatment of 300 MPa of 20 min, the parent strain (EGDe) showed 100-fold higher survival compared to σ^B mutant strain (Wemekamp-Kamphuis *et al.*, 2004a).

Apart from σ^B, other sigma factors σ^C, σ^H, and σ^L (RpoN) are also known to play important roles in stress adaptation of *L. monocytogenes*. *L. monocytogenes* strain lacking σ^B, σ^C, σ^H encoding proteins have been shown to have significantly impaired growth compared to wild type strain at 4°C for 12 days (Chan *et al.*, 2008). Raimann *et al.* (2009) reported that *L. monocytogenes* strain lacking σ^L has impaired cold growth due to in part by the repressed transcript production of oligopeptide-binding OppA protein that facilitates accumulation of short peptide substrates which are also important for efficient cold growth in this bacterium (Borezee *et al.*, 2000). Absence of σ^C increases the *L. monocytogenes* sensitivity to thermal treatment, thus highlighting the importance of this regulatory factor in conferring *L. monocytogenes* adaptation to heat stress (Zhang *et al.*, 2005). σ^L aids in *L. monocytogenes* ability to grow at high salt concentrations (Okada *et al.*, 2006) as well as control carbohydrate metabolism through its influence on expression of phosphotransferase system genes (Arous *et al.*, 2004).

CONCLUSION AND FUTURE PERSPECTIVES

The ability of *L. monocytogenes* cells to survive adverse physiological conditions is a serious food safety and public health concern. The physiological changes in response of environmental stress stimuli's reflect key changes instituted by microbial cells at gene or protein expression levels. In the future an improved understanding of fundamental changes occurring at genes or proteins level in *L. monocytogenes* cells in response to adverse environmental conditions will provides new insights that can be harnessed in developing more effective practical food preservation approaches (Gandhi and Chikindas, 2007; Tasara and Stephan, 2006).

The physiological changes mounted in response to particular environmental stress stimuli in *L. monocytogenes* are a consequence of changes at gene transcription and/or protein expression levels. The cold adaptive nature of this organism is probably one of the most important concerns to food production due to the ability of this pathogen to grow and achieve high concentrations in long shelf life ready-to-eat products preserved by refrigeration. Various cold adaptive mechanisms such as synthesis of conserved cold shock proteins (Schmid *et al.*, 2009), increased uptake of cryoprotective osmolytes (Angelidis and Smith, 2003), increased membrane permiablity (Borezee *et al.*, 2000), increased production of general stress proteins Fri (Dussurget *et al.*, 2005), etc have been identified that may directly or indirectly confer this bacterium with an ability to multiply and/or survive at lower temperatures. However, at this stage it is unclear if these different mechanisms work in any coordinated manner or if they work on separate niches leading overall cold stress resistance of *L. monocytogenes* cells. Future experiments are warranted to understand the complex hierarchy between these different stress response mechanisms. One way to do this would be to conduct gene knock out studies where the related genes/proteins of a particular stress adaptive mechanism (i.e. deletion of cold shock proteins) is deleted and use these strains to understand the modulations in genes/proteins of other stress mechanisms. The adaptation of this bac-

terium to osmotic stress also involves complex sets of cellular responses. Notably some osmotic stress response mechanisms, such as compatible solute uptake systems as well as alternative sigma factors are also involved in cold stress adaptation (Fraser *et al.*, 2003; Wemekamp-Kamphuis *et al.*, 2004b), which suggests that some cellular response mechanism towards food related environmental (cold and osmotic) stresses in this bacterium are common.

The main limitation of current studies is that large numbers of genes/proteins are tabulated as being differentially expressed but there is little or no insight on what the modulations in these gene/proteins mean. In any event, perturbation in physiology of living cells is likely to change the expression levels of various genes/proteins. Such information is of limited value without further functional characterizations of such putative stress adaptation genes or proteins. While it may not be practical to use such approach for hundreds of genes/proteins that are differentially expressed along with each stress, it is necessary to do follow-up studies on genes/proteins that exhibit substantially large changes in expression level. So far only in a few cases of stress proteins has the follow-up work been done in elucidating their molecular roles during stress adaptation of *L. monocytogenes*. Some key examples are: (a) Flp protein, first identified to be highly induced in cold and heat stress, and subsequently confirmed through *fri* mutant strain of *L. monocytogenes* EGDe, which is impaired under both stress conditions (Dussurget *et al.*, 2005; Hebraud and Guzzo, 2000; Phan-Thanh and Gormon, 1995); (b) Ctc protein is induced under salt stress and *L. monocytogenes* LO28 *ctc* mutant strain is found defective in growth under NaCl stress conditions (Duche *et al.*, 2002a; Gardan *et al.*, 2003b); and (c) GbuA osmolyte transporter protein, induced under high osmolarity, (at 3.5% NaCl) was confirmed by *gbu* mutant strain of *L. monocytogenes* LTG59 as defective in growth in the absence of osmolyte uptake activity (Duche *et al.*, 2002a; Mendum and Smith, 2002). Moreover most of the current stress adaptation findings are based on laboratory media and it is crucial that to design new experimental strategies that detect stress adaption response in *L. monocytogenes* cells exposed to different food matrices. The experiments with food substrate may be designed to see how different food components and food preservatives modulate the expression of stress proteins identified using broth media.

ACKNOWLEDGEMENT

This research was supported in part by Food Safety Initiative award to RN by the Mississippi Agricultural and Forestry Experiment Station (MAFES), Mississippi State University.

REFERENCES

Abram, F., E. Starr, K. A. Karatzas, K. Matlawska-Wasowska, A. Boyd, M. Wiedmann, K. J. Boor, D. Connally, C. P. O'Byrne. 2008a. Identification of components of the sigma B regulon in *Listeria monocytogenes* that contribute to acid and salt tolerance. Appl. Environ. Microbiol. 74:6848-6858.

Abram, F., W. L. Su, M. Wiedmann, K. J. Boor, P. Coote, C. Botting, K. A. Karatzas, C. P. O'Byrne. 2008b. Proteomic analyses of a *Listeria monocytogenes* mutant lacking sigmaB identify new components of the sigmaB regulon and highlight a role for sigmaB in the utilization of glycerol. Appl. Environ. Microbiol. 74:594-604.

Agoston, R., K. Soni, P. R. Jesudhasan, W. K. Russell, C. Mohacsi-Farkas, S. D. Pillai. 2009. Differential expression of proteins in *Listeria monocytogenes* under thermotolerance-inducing, heat shock, and prolonged heat shock conditions. Foodborne Pathog. Dis. 6:1133-1140.

Angelidis, A. S., L. T. Smith, G. M. Smith. 2002. Elevated carnitine accumulation by *Listeria monocytogenes* impaired in glycine betaine transport is insufficient to restore wild-type cryotolerance in milk whey. Int. J. Food Microbiol. 75:1-9.

Angelidis, A. S. and G. M. Smith. 2003. Role of the glycine betaine and carnitine transporters in adaptation of *Listeria monocytogenes* to chill stress in defined medium. Appl. Environ. Microbiol. 69:7492-7498.

Arous, S., C. Buchrieser, P. Folio, P. Glaser, A. Na-

mane, M. Hebraud, Y. Hechard. 2004. Global analysis of gene expression in an rpoN mutant of *Listeria monocytogenes*. Microbiol. 150:1581-1590.

Aymerich, T., A. Jofre, M. Garriga, M. Hugas. 2005. Inhibition of *Listeria monocytogenes* and *Salmonella* by natural antimicrobials and high hydrostatic pressure in sliced cooked ham. J. Food Prot. 68:173-177.

Bayles, D. O., B. A. Annous, B. J. Wilkinson. 1996. Cold stress proteins induced in *Listeria monocytogenes* in response to temperature downshock and growth at low temperatures. Appl. Environ. Microbiol. 62:1116-1119.

Becker, L. A., M. S. Cetin, R. W. Hutkins, A. K. Benson. 1998. Identification of the gene encoding the alternative sigma factor sigmaB from *Listeria monocytogenes* and its role in osmotolerance. J. Bacteriol. 180:4547-4554.

Becker, L. A., S. N. Evans, R. W. Hutkins, A. K. Benson. 2000. Role of sigma(B) in adaptation of *Listeria monocytogenes* to growth at low temperature. J. Bacteriol. 182:7083-7087.

Booth, I. R. and P. Louis. 1999. Managing hypoosmotic stress: aquaporins and mechanosensitive channels in *Escherichia coli*. Curr. Opin. Microbiol. 2:166-169.

Borezee, E., E. Pellegrini, P. Berche. 2000. OppA of *Listeria monocytogenes*, an oligopeptide-binding protein required for bacterial growth at low temperature and involved in intracellular survival. Infect. Immun. 68:7069-7077.

Cacace, G., M. F. Mazzeo, A . Sorrentino, V . Spada, A . Malorni, R. A Siciliano. 2010. Proteomics for the elucidation of cold adaptation mechanisms in *Listeria monocytogenes*. J. Proteomics 10:2021-2030.

Calvo, E., M. G. Pucciarelli, H. Bierne, P. Cossart, J. P. Albar, F. Garcia-Del Portillo. 2005. Analysis of the *Listeria* cell wall proteome by two-dimensional nanoliquid chromatography coupled to mass spectrometry. Proteomics 5:433-443.

Cetin, M. S., C. Zhang, R. W. Hutkins, A. K. Benson. 2004. Regulation of transcription of compatible solute transporters by the general stress sigma factor, sigmaB, in *Listeria monocytogenes*. J. Bacteriol. 186:794-802.

Chan, Y. C., Y. Hu, S. Chaturongakul, K. D. Files, B. M. Bowen, K. J. Boor, M. Wiedmann. 2008. Contributions of two-component regulatory systems, alternative sigma factors, and negative regulators to *Listeria monocytogenes* cold adaptation and cold growth. J. Food. Prot. 71:420-425.

Chan, Y. C., K. J. Boor, M. Wiedmann. 2007. SigmaB-dependent and sigmaB-independent mechanisms contribute to transcription of *Listeria monocytogenes* cold stress genes during cold shock and cold growth. Appl. Environ. Microbiol. 73:6019-6029.

Chassaing, D. and F. Auvray. 2007. The *lmo1078* gene encoding a putative UDP-glucose pyrophosphorylase is involved in growth of *Listeria monocytogenes* at low temperature. FEMS Microbiol. Lett. 275:31-37.

Chastanet, A., I. Derre, S. Nair, T. Msadek. 2004. *clpB*, a novel member of the *Listeria monocytogenes* CtsR regulon, is involved in virulence but not in general stress tolerance. J. Bacteriol. 186:1165-1674.

Christiansen, J. K., M. H. Larsen, H. Ingmer, L. Søgaard-Andersen, B. H. Kallipolitis. 2004. The RNA-binding protein Hfq of *Listeria monocytogenes* role in stress tolerance and virulence. J. Bacteriol. 186:3355-3362.

Conte, M.P, G. Petrone, A. M. Di Biase, M. G. Ammendolia, F. Superti, L. Seganti. 2000. Acid tolerance in *Listeria monocytogenes* influences invasiveness of enterocyte-like cells and macrophage-like cells. Microb. Pathog. 9:137-144.

Cotter P. D., C. G. Gahan, C. Hill. 2001a. A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. Mol. Microbiol. 40:465-475.

Cotter P. D., K.O'Reilly, C. Hill. 2001b. Role of the glutamate decarboxylase acid resistance system in the survival of *Listeria monocytogenes* LO28 in low pH foods. J. Food Prot. 64:1362-1368.

Davis, M. J., P. J. Coote, C. P. O'Byrne. 1996. Acid tolerance in *Listeria monocytogenes*: the adaptive acid tolerance response (ATR) and growth-phase-dependent acid resistance. Microbiology 142:2975-2982.

Donaldson, J. R., B. Nanduri, S. C. Burgess, M. L.

Lawrence. 2009. Comparative proteomic analysis of *Listeria monocytogenes* strains F2365 and EGD. Appl. Environ. Microbiol. 75:366-373.

Duche, O., F. Tremoulet, P. Glaser, J. Labadie. 2002a. Salt stress proteins induced in *Listeria monocytogenes*. Appl. Environ. Microbiol. 68:1491-1498.

Duche, O., F. Tremoulet, A. Namane, J. Labadie. 2002b. A proteomic analysis of the salt stress response of *Listeria monocytogenes*. FEMS Microbiol. Lett. 215:183-188.

Dumas, E., B. Meunier, J. L. Berdague, C. Chambon, M. Desvaux, M. Hebraud. 2008. Comparative analysis of extracellular and intracellular proteomes of *Listeria monocytogenes* strains reveals a correlation between protein expression and serovar. Appl. Environ. Microbiol. 74:7399-7409.

Dussurget, O., E. Dumas, C. Archambaud, I. Chafsey, C. Chambon, M. Hebraud, P. Cossart. 2005. *Listeria monocytogenes* ferritin protects against multiple stresses and is required for virulence. FEMS Microbiol. Lett. 250:253-261.

Duodu, S., A. Holst-Jensen, T. Skjerdal, J. M. Cappelier, M. F. Pilet, S. Loncarevic. 2010. Influence of storage temperature on gene expression and virulence potential of *Listeria monocytogenes* strains grown in a salmon matrix. Food Microbiol. 27:795-801.

Esvan, H., J. Minet, C. Laclie, M. Cormier. 2000. Proteins variations in *Listeria monocytogenes* exposed to high salinities. Int. J. Food Microbiol. 55:151-155.

Ferreira, A., D. Sue, C. P. O'Byrne, K. J. Boor. 2003. Role of *Listeria monocytogenes* sigma(B) in survival of lethal acidic conditions and in the acquired acid tolerance response. Appl. Environ. Microbiol. 69:2692-2698.

Folio, P., P. Chavant, I. Chafsey, A. Belkorchia, C. Chambon, M. Hebraud. 2004. Two-dimensional electrophoresis database of *Listeria monocytogenes* EGDe proteome and proteomic analysis of mid-log and stationary growth phase cells. Proteomics 4:3187-3201.

Folsom, J. P. and J. F. Frank. 2007. Proteomic analysis of a hypochlorous acid-tolerant *Listeria monocytogenes* cultural variant exhibiting enhanced biofilm production. J. Food Prot. 70:1129-1136.

Foster, J. W. 2004. *Escherichia coli* acid resistance: tales of an amateur acidophile. Nat. Rev. Microbiol. 2:898-907.

Fraser, K. R., D. Sue, M. Wiedmann, K. Boor, C. P. O'Byrne. 2003. Role of sigmaB in regulating the compatible solute uptake systems of *Listeria monocytogenes*: osmotic induction of opuC is sigmaB dependent. Appl. Environ. Microbiol. 69:2015-2022.

Gahan, C. G., J. O'Mahony J, C. Hill. 2001 .Characterization of the GroESL operon in *Listeria monocytogenes*: utilization of two reporter systems (*gfp* and *hly*) for evaluating in vivo expression. Infect. Immun. 69:3924-3932.

Gaillot, O., E. Pellegrini, S. Bregenholt, S. Nair, P. Berche. 2000. The ClpP serine protease is essential for the intracellular parasitism and virulence of *Listeria monocytogenes*. Mol. Microbiol. 35:1286-1294.

Gandhi, M., and M. L. Chikindas. 2007. *Listeria*: A foodborne pathogen that knows how to survive. Int. J. Food Microbiol. 113:1-15.

Gardan, R., P. Cossart, J. Labadie. 2003a. Identification of *Listeria monocytogenes* genes involved in salt and alkaline-pH tolerance. Appl. Environ. Microbiol. 69:3137-3143.

Gardan, R., O. Duche, S. Leroy-Setrin, J. Labadie. 2003b. Role of *ctc* from *Listeria monocytogenes* in osmotolerance. Appl. Environ. Microbiol. 69:154-161.

Garner, M. R., K. E. James, M. C. Callahan, M. Wiedmann, K. J. Boor. 2006. Exposure to salt and organic acids increases the ability of *Listeria monocytogenes* to invade Caco-2 cells but decreases its ability to survive gastric stress. Appl. Environ. Microbiol. 72:5384-5395.

Giotis, E. S., D. A. McDowell, I. S. Blair, B. J. Wilkinson. 2007. Role of branched-chain fatty acids in pH stress tolerance in *Listeria monocytogenes*. Appl. Environ. Microbiol. 73:997-1001.

Giotis, E. S., A. Muthaiyan, I. S. Blair, B. J. Wilkinson, D. A. McDowell. 2008. Genomic and proteomic analysis of the Alkali-Tolerance Response (AlTR) in *Listeria monocytogenes* 10403S. BMC Microbiol. 8:102.

Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok,

A. Amend, F. Baquero, P. Berche, H. Bloecker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couve, A. de Daruvar, P. Dehoux, E. Domann, G. Dominguez-Bernal, E. Duchaud, L. Durant, O. Dussurget, K. D. Entian, H. Fsihi, F. Garcia-del Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gomez-Lopez, T. Hain, J. Hauf, D. Jackson, L. M. Jones, U. Kaerst, J. Kreft, M. Kuhn, F. Kunst, G. Kurapkat, E. Madueno, A. Maitournam, J. M. Vicente, E. Ng, H. Nedjari, G. Nordsiek, S. Novella, B. de Pablos, J. C. Perez-Diaz, R. Purcell, B. Remmel, M. Rose, T. Schlueter, N. Simoes, A. Tierrez, J. A. Vazquez-Boland, H. Voss, J. Wehland, P. Cossart. 2001. Comparative genomics of *Listeria* species. Science 294:849-852.

Gross, M. and R. Jaenicke. 1994. Proteins under pressure. The influence of high hydrostatic pressure on structure, function and assembly of proteins and protein complexes. Europ. J. Biochem. 221:617-630.

Hanawa, T., M. Kai, S, Kamiya, T. Yamamoto. 2000. Cloning, sequencing, and transcriptional analysis of the dnaK heat shock operon of *Listeria monocytogenes*. Cell Stress Chaperones 5:21-29.

Haynes, P. A. and T. H. Roberts. 2007. Subcellular shotgun proteomics in plants: looking beyond the usual suspects. Proteomics 7:2963-2975.

Hebraud, M. and J. Guzzo. 2000. The main cold shock protein of *Listeria monocytogenes* belongs to the family of ferritin-like proteins. FEMS Microbiol. Lett. 190:29-34.

Horn, G., R. Hofweber, W. Kremer, H. R. Kalbitzer. 2007. Structure and function of bacterial cold shock proteins. Cell Mol. Life Sci. 64: 1457-1470.

Hu, Y., H. F. Oliver, S. Raengpradub, M. E. Palmer, R. H. Orsi, M. Wiedmann, K. J. Boor. 2007a. Transcriptomic and phenotypic analyses suggest a network between the transcriptional regulators HrcA and sigmaB in *Listeria monocytogenes*. Appl. Environ. Microbiol. 73:7981-7991.

Hu, Y., S. Raengpradub, U. Schwab, C. Loss, R. H. Orsi, M. Wiedmann, K. J. Boor. 2007b. Phenotypic and transcriptomic analyses demonstrate interactions between the transcriptional regulators CtsR and Sigma B in *Listeria monocytogenes*. Appl. Environ. Microbiol. 73:7967-7980.

Ivy, R. A, Y. C. Chan, B. M. Bowen, K. J. Boor, M. Wiedmann. 2010. Growth temperature-dependent contributions of response regulators, B, PrfA, and motility factors to *Listeria monocytogenes* invasion of Caco-2 cells. Foodborne Pathog. Dis. 11:1337-1349.

Jensen, A., M. H. Larsen, H. Ingmer, B. F. Vogel, L. Gram. 2007. Sodium chloride enhances adherence and aggregation and strain variation influences invasiveness of *Listeria monocytogenes* strains. J. Food Prot. 70:592-599.

Jensen, A., L. E. Thomsen, R. L. Jørgensen, M. H. Larsen, B. B. Roldgaard, B. B. Christensen, B. F. Vogel, L. Gram, H. Ingmer. 2008. Processing plant persistent strains of *Listeria monocytogenes* appear to have a lower virulence potential than clinical strains in selected virulence models. Int. J. Food Microbiol. 23:254-261

Jofre, A., M. Champomier-Verges, P. Anglade, F. Baraige, B. Martin, M. Garriga, M. Zagorec, T. Aymerich. 2007. Protein synthesis in lactic acid and pathogenic bacteria during recovery from a high pressure treatment. Res. Microbiol. 158:512-520.

Johansson, J., P. Mandin, A. Renzoni, C. Chiaruttini, M. Springer, P. Cossart. 2002. An RNA thermosensor controls the expression of virulence genes in *Listeria monocytogenes*. Cell 110:551-561.

Kandror, O., L. Busconi, M. Sherman, A. L. Goldberg. 1994. Rapid degradation of an abnormal protein in *Escherichia coli* involves the chaperones GroEL and GroES. The J. Biol. Chem. 269: 75-82.

Kazmierczak, M. J., S. C. Mithoe, K. J. Boor, M. Wiedmann. 2003. *Listeria monocytogenes* sigma B regulates stress response and virulence functions. J. Bacteriol. 185:5722-5734.

Klinkert B. and F. Narberhaus. 2009. Microbial thermosensors. Cell Mol. Life Sci. 66:2661-2676.

Koutsoumanis, K. P. and J. N. Sofos. 2004. Comparative acid stress response of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium after habituation at different pH conditions. Lett. Appl. Microbiol. 38:321-326.

Ko, R. and L. T. Smith. 1999. Identification of an ATP-driven, osmoregulated glycine betaine transport

system in *Listeria monocytogenes*. Appl. Environ. Microbiol. 65:4040-4048.

Kramer, M. N., D. Coto, J. D. Weidner. 2005. The science of recalls. Meat Sci. 71:158–163.

Lennon, D, B. Lewis, C. Mantell, D. Becroft, B. Dove, K. Farmer, S. Tonkin, N. Yeates, R. Stamp, K. Mickleson. 1984. Epidemic perinatal listeriosis. Pediatric Infect. Dis. 3:30-34.

Liu S., J. E. Graham, L. Bigelow, P. D. Morse 2nd, B. J. Wilkinson. 2002. Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Appl. Environ. Microbiol. 68:1697-1705

Liu. S., D. O. Bayles, T. M. Mason, B. J. Wilkinson. 2006. A cold-sensitive *Listeria monocytogenes* mutant has a transposon insertion in a gene encoding a putative membrane protein and shows altered (p)ppGpp levels. Appl. Environ. Microbiol. 72:3955-3959.

Loepfe, C., E. Raimann, R. Stephan, T. Tasara. 2010. Reduced host cell invasiveness and oxidative stress tolerance in double and triple *csp* gene family deletion mutants of *Listeria monocytogenes*. Foodborne Pathog. Dis. 7:775-783.

Lou, Y. and A. E. Yousef. 1997. Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. Appl. Environ. Microbiol. 63:1252-1255.

Marsden, J. L., R.K. Phebus, H. Thippareddi. 2001. *Listeria* risks in ready-to-eat meats can be controlled using a true HACCP approach. in Scientific Conference, USDA Public Meetings on the FSIS Proposed Rule, "Performance Standards for the Production of Processed Meat and Poultry Products." Available at http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/97-013N/JMarsden_97-013N.pdf.

Mbandi, E., B. S. Phinney, D. Whitten, L. A. Shelef. 2007. Protein variations in *Listeria monocytogenes* exposed to sodium lactate, sodium diacetate, and their combination. J. Food Prot. 70:58-64.

McClure, P. J., T. M. Kelly, T. A. Roberts. 1991. The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. Int. J. Food Microbiol. 14:77-91.

McGann, P., M. Wiedmann, K. J. Boor. 2007. The alternative sigma factor σ^B and the virulence gene regulator PrfA both regulate transcription of *Listeria monocytogenes* internalins. Appl. Environ. Microbiol. 73: 2919-2930.

Mendonca, A. F., T. L. Amoroso, S. J. Knabel. 1994. Destruction of gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. Appl. Environ. Microbiol. 60:4009-4014.

Mendum, M. L. and L. T. Smith. 2002. Characterization of glycine betaine porter I from *Listeria monocytogenes* and its roles in salt and chill tolerance. Appl. Environ. Microbiol. 68:813-819.

Mohamed, W., A. Darji, E. Domann, E. Chiancone, T. Chakraborty. 2006. The ferritin-like protein Frm is a target for the humoral immune response to *Listeria monocytogenes* and is required for efficient bacterial survival. Mol. Genet. Genomics 275:344-353.

Merrell D, S. and A. Camilli. 2002. Acid tolerance of gastrointestinal pathogens. Curr. Opin. Microbiol. 5:51-55.

Moorhead, S. M. and G. A. Dykes. 2004. Influence of the sigB gene on the cold stress survival and subsequent recovery of two *Listeria monocytogenes* serotypes. Int. J. Food Microbiol. 91:63-72.

Muga, A. and F. Moro. 2008. Thermal adaptation of heat shock proteins. Curr. Protein Pept. Sci. 9:552-566.

Mujahid, S., T. Pechan, C. Wang. 2007. Improved solubilization of surface proteins from *Listeria monocytogenes* for 2-DE. Electrophoresis 28:3998-4007.

Mujahid, S., T. Pechan, C. Wang. 2008. Protein expression by *Listeria monocytogenes* grown on a RTE-meat matrix. Int. J. Food Microbiol. 128:203-211.

Nair, S., C. Frehel, L. Nguyen, V. Escuyer, P. Berche. 1999. ClpE, a novel member of the HSP100 family, is involved in cell division and virulence of *Listeria monocytogenes*. Mol. Microbiol. 31:185-196.

Nair, S., E. Milohanic, P. Berche. 2000a. ClpC ATPase is required for cell adhesion and invasion of *Listeria monocytogenes*. Infect. Immun. 68:7061-7068.

Nair, S., I. Derre, T. Msadek, O. Gaillot, P. Berche. 2000b. CtsR controls class III heat shock gene expression in the human pathogen *Listeria monocytogenes*. Mol. Microbiol. 35:800-811.

Nesatyy, V. J. and M. J. Suter. 2007. Proteomics for

the analysis of environmental stress responses in organisms. Environ. Sci. Technol. 41:6891-6900.

O'Driscoll, B., C. Gahan, C. Hill. 1997. Two-dimensional polyacrylamide gel electrophoresis analysis of the acid tolerance response in *Listeria monocytogenes* LO28. Appl. Environ. Microbiol. 63:2679-2685.

Okada. Y., N. Okada, S. Makino, H. Asakura, S. Yamamoto, S. Igimi S. 2006. The sigma factor RpoN (sigma54) is involved in osmotolerance in *Listeria monocytogenes*. FEMS Microbiol. Lett. 263:54-60.

Okada, Y., S. Makino, N. Okada, H. Asakura, S. Yamamoto, S. Igimi. 2008. Identification and analysis of the osmotolerance associated genes in *Listeria monocytogenes*. Food Addit. Contam. 15:1-6.

Olsen, K. N., M. H. Larsen, C. G. Gahan, B. Kallipolitis, X. A. Wolf, R. Rea, C. Hill, H. Ingmer. 2005. The Dps-like protein Fri of *Listeria monocytogenes* promotes stress tolerance and intracellular multiplication in macrophage-like cells. Microbiology 151:925-933.

Olesen, I., F. K. Vogensen, L. Jespersen. 2009. Gene transcription and virulence potential of *Listeria monocytogenes* strains after exposure to acidic and NaCl stress. Foodborne Pathog. Dis. 6:669-680.

Oliver, H. F., R. H. Orsi, M. Wiedmann, K. J. Boor. 2010. *Listeria monocytogenes* {sigma}B has a small core regulon and a conserved role in virulence but makes differential contributions to stress tolerance across a diverse collection of strains. Appl. Environ. Microbiol. 76:4216-4232.

Pan, Y., F. Breidt, Jr., S. Kathariou. 2006. Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. Appl. Environ. Microbiol. 72:7711-7717.

Phan-Thanh, L. and T. Gormon. 1995. Analysis of heat and cold shock proteins in *Listeria* by two-dimensional electrophoresis. Electrophoresis 16:444-450.

Phan-Thanh, L. and T. Gormon. 1997. Stress proteins in *Listeria monocytogenes*. Electrophoresis 18:1464-1471.

Phan-Thanh, L. and L. Jansch. 2006. Elucidation of mechanisms of acid stress in *Listeria monocytogenes* by proteomic analysis. Methods. Biochem. Anal. 49:75-88.

Phan-Thanh, L. and F. Mahouin. 1999. A proteomic approach to study the acid response in *Listeria monocytogenes*. Electrophoresis 20:2214-2224.

Pucciarelli, M. G., E. Calvo, C. Sabet, H. Bierne, P. Cossart, F. Garcia-del Portillo. 2005. Identification of substrates of the *Listeria monocytogenes* sortases A and B by a non-gel proteomic analysis. Proteomics 5:4808-4817.

Raimann, E., B. Schmid, R. Stephan, T. Tasara. 2009. The alternative sigma factor sigma(L) of *L. monocytogenes* promotes growth under diverse environmental stresses. Foodborne Pathog. Dis. 6:583-591.

Ramnath, M., K. B. Rechinger, L. Jansch, J. W. Hastings, S. Knochel, A. Gravesen. 2003. Development of a *Listeria monocytogenes* EGDe partial proteome reference map and comparison with the protein profiles of food isolates. Appl. Environ. Microbiol. 69:3368-3376.

Ritz, M., M. F. Pilet, F. Jugiau, F. Rama, M. Federighi. 2006. Inactivation of *Salmonella* Typhimurium and *Listeria monocytogenes* using high-pressure treatments: destruction or sublethal stress? Lett. Appl. Microbiol. 42:357-362.

Ryan, E. M., C. G. Gahan, C. Hill. 2008a. A significant role for SigmaB in the detergent stress response of *Listeria monocytogenes*. Lett. Appl. Microbiol. 46:148-154.

Ryan, S., C. Hill, C. G. Gahan. 2008b. Acid stress responses in *Listeria monocytogenes*. Adv. Appl. Microbiol. 65:67-91.

Ryan, S., M. Begley, C. G. Gahan, C. Hill. 2009. Molecular characterization of the arginine deiminase system in *Listeria monocytogenes*: regulation and role in acid tolerance. App. Environ. Microbiol. 11:432-445.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, P. M. Griffin. 2011. Foodborne illness acquired in the United States--major pathogens. Emerg. Infect. Dis. 17:7-15.

Schaumburg, J., O. Diekmann, P. Hagendorff, S. Bergmann, M. Rohde, S. Hammerschmidt, L. Jansch, J. Wehland, U. Karst. 2004. The cell wall

subproteome of *Listeria monocytogenes*. Proteomics 4:2991-3006.

Schmid, B, J. Klumpp, E. Raimann, M. J. Loessner, R. Stephan, T. Tasara. 2009. Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. Appl. Environ. Microbiol. 75:1621-1627.

Schumann, W. 2009. Temperature sensors of eubacteria. Adv. Appl. Microbiol. 67:213-256.

Sherman, M. Y. and A. L. Goldberg. 1996. Involvement of molecular chaperones in intracellular protein breakdown. Exs. 77:57-78.

Shin, J. H., J. Kim, S. M. Kim, S. Kim, J. C. Lee, J. M. Ahn, J. Y. Cho. 2010. SigmaB-dependent protein induction in *Listeria monocytogenes* during vancomycin stress. FEMS Microbiol Lett. 308:94-100.

Skandamis, P. N., Y. Yoon, J. D. Stopforth, P. A. Kendall, J. N. Sofos. 2008. Heat and acid tolerance of *Listeria monocytogenes* after exposure to single and multiple sublethal stresses. Food Microbiol. 25:294-303.

Sleator, R. D., C. G. Gahan, T. Abee, C. Hill. 1999. Identification and disruption of BetL, a secondary glycine betaine transport system linked to the salt tolerance of *Listeria monocytogenes* LO28. Appl. Environ. Microbiol. 65:2078-2083.

Smith L, T. 1996. Role of osmolytes in adaptation of osmotically stressed and chill-stressed *Listeria monocytogenes* grown in liquid media and on processed meat surfaces. Appl. Environ. Microbiol. 62:3088-93.

Sokolovic, Z., A. Fuchs, W. Goebel. 1990. Synthesis of species-specific stress proteins by virulent strains of *Listeria monocytogenes*. Infect. Immu. 58:3582-3587.

Stack, H. M., R. D. Sleator, M. Bowers, C. Hill, C. G. Gahan. 2005. Role for HtrA in stress induction and virulence potential in *Listeria monocytogenes*. Appl. Environ. Microbiol. 71:4241-4247.

Sue, D., D. Fink, M. Wiedmann, K. J. Boor. 2004. SigmaB-dependent gene induction and expression in *Listeria monocytogenes* during osmotic and acid stress conditions simulating the intestinal environment. Microbiology 150:3843-3855.

Tasara, T. and R. Stephan. 2006. Cold stress tolerance of *Listeria monocytogenes*: A review of molecular adaptive mechanisms and food safety implications. J. Food Prot. 69:1473-1484.

Teratanavat, R. and N.H. Hooker. 2004. Understanding the characteristics of US meat and poultry recalls: 1994–2002. Food Control 15:359–367.

Trost, M., D. Wehmhoner, U. Karst, G. Dieterich, J. Wehland, L. Jansch. 2005. Comparative proteome analysis of secretory proteins from pathogenic and nonpathogenic *Listeria* species. Proteomics 5:1544-1557.

van der Veen, S., T. Hain, J. A. Wouters, H. Hossain, W. M. de Vos, T. Abee, T. Chakraborty, M. H. Wells-Bennik. 2007. The heat-shock response of *Listeria monocytogenes* comprises genes involved in heat shock, cell division, cell wall synthesis, and the SOS response. Microbiology 153:3593-3607.

Van de Velde, S., E. Delaive, M. Dieu, S. Carryn, F. Van Bambeke, B. Devreese, M. Raes, P. M. Tulkens. 2009. Isolation and 2-D-DIGE proteomic analysis of intracellular and extracellular forms of *Listeria monocytogenes*. Proteomics 9:5484-5496.

Wałecka, E., J. Molenda, R. Karpíšková, J. Bania. 2011. Effect of osmotic stress and culture density on invasiveness of *Listeria monocytogenes* strains. Int. J. Food Microbiol. 144:440-445.

Wehmhoner, D., G. Dieterich, E. Fischer, M. Baumgartner, J. Wehland, L. Jansch. 2005. "Lane-Spector", a tool for membrane proteome profiling based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis/liquid chromatography-tandem mass spectrometry analysis: application to *Listeria monocytogenes* membrane proteins. Electrophoresis 26:2450-2460.

Wemekamp-Kamphuis, H. H., A. K. Karatzas, J. A. Wouters, T. Abee. 2002. Enhanced levels of cold shock proteins in *Listeria monocytogenes* LO28 upon exposure to low temperature and high hydrostatic pressure. Appl. Environ. Microbiol. 68:456-463.

Wemekamp-Kamphuis, H. H., J. A. Wouters, P. P. de Leeuw, T. Hain, T. Chakraborty, T. Abee. 2004a. Identification of sigma factor sigma B-controlled genes and their impact on acid stress, high hydrostatic pressure, and freeze survival in *Listeria*

monocytogenes EGD-e. Appl. Environ. Microbiol. 70:3457-3466.

Wemekamp-Kamphuis, H. H., R. D. Sleator, J. A. Wouters, C. Hill, T. Abee. 2004b. Molecular and physiological analysis of the role of osmolyte transporters BetL, Gbu, and OpuC in growth of *Listeria monocytogenes* at low temperatures. Appl. Environ. Microbiol. 70:2912-2918.

Werbrouck, H., A. Vermeulen, E. Van Coillie, W. Messens, L. Herman, F. Devlieghere, M. Uyttendaele. 2009. Influence of acid stress on survival, expression of virulence genes and invasion capacity into Caco-2 cells of *Listeria monocytogenes* strains of different origins. Int. J. Food Microbiol. 134:140-146.

Wonderling, L. D., B. J. Wilkinson, D. O. Bayles. 2004. The *htrA* (degP) gene of *Listeria monocytogenes* 10403S is essential for optimal growth under stress conditions. Appl. Environ. Microbiol. 70:1935-1943.

Wood, J. M. 2007. Bacterial osmosensing transporters. Methods Enzymol. 428:77-107.

Zhang, C., J. Nietfeldt, M. Zhang, A. K. Benson. 2005. Functional consequences of genome evolution in *Listeria monocytogenes*: the *lmo0423* and *lmo0422* genes encode sigmaC and LstR, a lineage II-specific heat shock system. J. Bacteriol. 187:7243-7253.